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Effect of Storage on the Content of Polyphenols of Minimally Processed Skin-On Apple Wedges from Ten Cultivars and Two Growing Seasons

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In this study, the polyphenolic composition of skin-on apple wedges from ten cultivars was examined during chill storage and over two growing seasons. Individual polyphenol compounds were measured using HPLC resulting in the total polyphenolic index (TPI). Total phenolic content (TPC) was quantified using the Folin−Ciocalteu assay. Chilled storage had a significant effect ($P < 0.001$) on the polyphenol composition of all ten cultivars grown in 2007 and 2008. Total phenolic indices (sum of individual polyphenols) and TPCs of nine of the ten cultivars significantly decreased ($P < 0.001$) after 5 days of storage at 2−4 °C. These indices increased in case of Shampion apples over the same storage period. Changes in the most abundant compounds (−)-epicatechin, procyanidins and chlorogenic acid were largely responsible for changes in overall TPI. Percentage loss was higher for compounds such as phloridzin with a degradation of up to 100%. Irrespective of the different starting level of specific polyphenols in each year; storage resulted in a similar percentage loss/gain for each cultivar.

KEYWORDS: Polyphenols; phytochemicals; fresh-cut; apple; fruit salad; chill storage

INTRODUCTION

In recent years interest in the phytochemical compositions of fruits has increased dramatically. Tree fruits such as apples have been shown to be a good source of polyphenolic compounds such as chlorogenic acid, procyanidins, catechins, phloridzin, quercetin and many more (*1*). Polyphenols in apples fall into five major groups, i.e., hydroxycinnamic acids, flavan-3-ols, flavonols, dihydrochalcones and anthocyanins (*2*, *3*). Evidence is accumulating that many polyphenols included in these groups confer health benefits on to the consumer. For example, a decreased risk of prostate, liver, colon and lung cancer and cardiovascular diseases has been associated with a high intake of apples (*4*−*6*). These beneficial effects may derive from the antioxidant and anti-inflammatory activities (*7*, *8*) of polyphenols in apples as these compounds represent the major antioxidant group present (*9*). While the nutritional significance of polyphenols is well recognized, polyphenols may not be beneficial and do play an undesirable role in apples, as substrates of polyphenol oxidase, and therefore are a source of enzymatic browning.

Polyphenolics also play a well-known role in the quality of fruits as they serve to protect them from oxidative deterioration (*10*).The composition and concentration of polyphenols is in turn dependent on factors such as cultivar, postharvest handling, fruit maturity, environmental factors, storage period and processing (*11*−*15*).

Traditionally apples are consumed whole or used in beverage and dessert products. However, recently the minimally processed foods market has expanded into the fresh-cut fruit market (*16*) and skin-on apples wedges are a core ingredient in products such as fresh-cut fruit salads (*17*, *18*). The production of these products is relatively simple and is usually based on sequential washing, cutting, packaging and refrigeration (*19*). Despite the known fact that minimal processing can affect the quality and quantity of polyphenols in foods (*14*, *20*), very little information is available on the effect of storage of fresh-cut fruits on their polyphenolic composition. This is despite the fact that fresh cut salad products are usually retailed in chill cabinets and stored for short periods (up to 5 days) before they pass their "display by" date. Therefore, the main aim of this study was to monitor quantitative and qualitative changes in polyphenolic compounds in apple slices similar to those used in fresh-cut fruit salads after slicing and during chill storage for 5 days at 2−4 °C. In order to confirm any effects observed ten apple cultivars ('Shampion', 'Jonica', 'Gloster', 'Topaz', 'Ariwa', 'Rajka', 'Idared', 'Cortland', 'Alwa' and 'Braeburn') from two growing seasons (2007 and 2008) were examined in this study.

MATERIALS AND METHODS

Chemicals. Procyanidin A2, procyanidin B1, procyanidin B2, ethyl gallate, 3-coumaric acid, 4-hydroxybenzoic acid, protocatechuic acid,

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sinapic acid, vanillic acid, apigenin, luteolin and kaempferol-3-*O*-glucoside were purchased from Extrasynthèse (Lyon, France). (+)-Catechin, cinnamic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, ellagic acid, ferulic acid, gallic acid, (−)-epicatechin, kaempferol, myricetin, quercetin, rutin, phloridzin and Folin−Ciocalteu Reagent (FCR) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

Preparation of Samples. Apple cultivars 'Shampion', 'Jonica', 'Gloster', 'Topaz', 'Ariwa', 'Rajka', 'Idared', 'Cortland' and 'Alwa' were all sourced from an ISAFRUIT project partner in Poland, and 'Braeburn' was purchased in Ireland. All apples were washed in water, cored using a 20 mm diameter stainless steel cork borer, and cut with a stainless-steel knife into wedges (each ca. 10 g). Apple wedges were used in this trial as they represent the same shape used in current fresh-cut fruit products. Two skin-on wedges from each of eight apples (chosen at random) were packed in clear trays (15 cm × 10.5 cm × 3 cm; Versatile Packaging, Silverstream, Ireland), covered (heat-sealed) with a breathing film (O_2 transmission <2 mL 24 h^{-1} at 23 °C; water vapor transmission < 6 g 24 h^{-1} at 38 °C) using a modified atmosphere packaging machine (Ilpra Foodpack Basic V/G, Ilpra, Vigenovo, Italy) and stored at 2−4 °C for 5 days. On each test day (days 0 and 5) all samples were vacuum packed and stored at −20 °C for at least 24 h. Following this, samples were removed from the vacuum pack and lyophilized for a minimum of 5 days in an A6/14 freeze-dryer (Frozen in Time Ltd., York, U.K.). The lyophilized samples were then vacuum packed and stored in the dark at −20 °C until required for analysis.

Extraction of Polyphenols. Each sample was extracted in duplicate. On the day of extraction, samples were milled to a fine powder using a blender (BL440001, Kenwood limited, Hampshire, U.K.). After addition of 25 mL of methanol to 1.25 g of sample powder, the samples were homogenized for 70 s at 24,000 rpm using an Ultra-Turrax T-25 tissue homogenizer (IKA-group, Saufen, Germany). The samples were vortexed with a V400 Multitube Vortexer (Alpha Laboratories, North York, Canada) for 20 min at 1050 rpm and centrifuged for 10 min at 2,000 rpm (MSE Mistral 3000i, Sanyo Gallenkamp, Leicestershire, U.K.). 10 mL of the supernatant was filtered through 0.22 μm PTFE syringe filters (Phenomenex, Macclesfield Cheshire, U.K.). All extracts were stored in the dark at −20 °C until required for analysis

HPLC-DAD Analysis of Polyphenolic Composition. HPLC analysis was performed on a SPD-M10A vp Shimadzu chromatographic system (Shimadzu UK Ltd., Milton Keynes, U.K.) equipped with pump, degasser and diode array detector (DAD) and controlled through EZ Start 7.3 software (Shimadzu UK Ltd., Milton Keynes, U.K.). Separations were conducted on a Zorbax SB C_{18}, 5 μm, 150 × 4.6 mm column (Agilent Technologies, Dublin, Ireland). The mobile phase consisted of 6% acetic acid in 2 mM sodium acetate (final pH 2.55, v/v, eluent A) and 100% acetonitrile (eluent B), and was based on the method described by Tsao and Yang (*21*). The solvent gradient program was as follows: initial conditions 95% A, 5% B; 0−45 min, 0−15% B; 45−60 min, 15−30% B; 60−65 min, 30−50% B; 65−70 min, 50−100% B. Column temperature was set at 37 °C, flow rate was 1 mL min^{-1} and the injection volume was 10 μL. As recommended by Tsao and Yang (*21*) hydroxybenzoic acids, dihydrochalcones, flavanones and flavonols were monitored at a wavelength of 280 nm, hydroxycinnamic acid derivatives at 320 nm, flavonols at 360 nm and anthocyanins at 520 nm. For identification purposes, a spectral library was constructed comprising the retention times and light absorbing spectra (200−900 nm) of authenticated standards under the chromatographic conditions specified above. For quantification purposes standard curves of authenticated standards were also prepared and results were expressed as μmol of aglycon/100 g dry sample. Compounds with the same spectrum as a library standard but with different retention times were referred to as derivatives of the standard and quantified using the respective standard calibration curve. Levels of compounds identified belonging to the four main groups of polyphenols were summed and are referred to as total hydroxycinnamic acids, total flavonols, total flavan-3-ols and total dihydrochalcones. Total phenolic index (TPI) represents the sum of all four main groups.

Measurement of Total Phenolics. Total phenolic content (TPC) of methanolic apple extracts was assessed using a modified version of the Folin−Ciocalteu (FC) assay (*22*). Gallic acid was used as a standard and the aqueous gallic acid solution (200 mg L^{-1}) was diluted with distilled water to give appropriate concentrations for a standard curve. For the analysis, 100 μL of methanolic fruit extract or gallic acid standard, 100 μL of methanol, 100 μL of Folin−Ciocalteu reagent and 700 μL of Na_2CO_3 were added into a 1.5 mL microcentrifuge tube. The samples were vortexed immediately, and the tubes were incubated in the dark for 20 min at room temperature. After incubation all samples were centrifuged at 13,000 rpm for 3 min. The absorbance of the supernatant was then measured at 735 nm in 1 mL plastic cuvettes using a spectrophotometer (UV-1700 Pharma Spec, Shimadzu, Japan). The results are expressed in mg of gallic acid equivalent/100 g dry weight (mg GAE 100 g^{-1} DW).

Determination of Moisture for Freeze-Dried Samples. For calculation of dry weight of freeze-dried samples, moisture contents were measured using 3 g of freeze-dried sample powder. Samples were dried in a vacuum oven (OVA031, Gallenkamp, Loughborough, U.K.) to a constant weight at 70 °C and 58 kPa overnight (approximately 18 h). The moisture content was determined by weight difference of the initial weight (*23*) and was used to calculate the dry weight.

Statistical Design. The following statistical design was used: 10 cultivars ('Shampion', 'Jonica', 'Gloster', 'Topaz', 'Ariwa', 'Rajka', 'Idared', 'Cortland', 'Alwa' and 'Braeburn') × 2 test days (0, 5) × 2 seasons (2007, 2008) × 2 extracts × 2 replicates with 159 degrees of freedom (df). ANOVA was carried out to determine significant differences between storage [(Genstat 5 version 3.2); Lawes Agricultural Trust, Rothamsted, Harpenden, U.K.].

RESULTS AND DISCUSSION

HPLC-DAD Analysis. The major polyphenolic compounds quantified by HPLC of all apple cultivars for the two storage days (0 and 5) are presented in **Table 1** (2007 season) and **Table 2** (2008 season). The nine phenolic compounds quantified are categorized into four groups: chlorogenic acid, *p*-coumaric acid and caffeic acid (hydroxycinnamic acids); (+)-catechin, (−)-epicatechin, procyanidin B1 and B2 (flavan-3-ols); quercetin-3-rutinoside (flavonols); phloridzin (dihydrochalchones). Other compounds which were also detected and exhibited absorption spectra similar to those are referred to as derivatives or glycosides. A representative chromatogram of the cultivar Braeburn (day 0, 2008) is shown as an example (Figure **2**).

In both growing seasons (2007 and 2008) flavan-3-ols were the most dominant group in apple skin-on wedges averaged from all cultivars and both storage days (0 and 5). In fact this group contributed on average 71% ± 4.3% to the TPI. Hydroxycinnamic acids (22 ± 2.6%) were the second most abundant group, followed by flavonols (5 ± 0.6%) and dihydrochalcones (3 ± 0.6%), respectively. A similar relative abundance of these groups has been reported by other authors (*24*, *25*). Anthocyanins were not detected in any of the samples. Several studies have shown that anthocyanins are only present in the apple peel (*26*, *27*). In the present study the peel of the apple wedge comprised approximately 5% of the total volume, and therefore the concentration was probably too low to be detectable. All cultivars except for Idared had a mainly greenish skin color.

In both seasons chill storage for 5 days at 2−4 °C of the apple wedges resulted in a significant reduction in TPIs for nine of the ten cultivars ($P < 0.001$), while this parameter increased for Shampion apples over the same storage period. The mean percentage loss/gain of hydroxycinnamic acids, flavan-3-ols, flavonols and dihydrochalcones over 5 days of storage at 2−4 °C for all ten cultivars over the two seasons are illustrated in **Figure 1**. It is interesting to note that irrespective of the differences in starting TPC between apples wedges from 2007 and 2008, chill storage resulted in a similar percentage loss/gain for each cultivar in both years. A reduction in levels of polyphenols is not surprising as cutting of whole apples exposes these compounds to oxygen and light (*28*) and releases polyphenolic degradation enzymes such as polyphenol oxidase (PPO) (*29*). This effect has been reported elsewhere in apples (*30*) and mango fruits (*31*).

Table 1. Concentrations (in μmol 100 g^{-1} DW) of Phenolic Compounds in Skin-On Apple Wedges from 10 Cultivars on Day 0 and after 5 Days of Storage at 2−4 °C in the Growing Season 2007[a]

	Alwa		Ariwa		Braeburn		Cortland		Gloster		Idared		Jonica		Rajka		Shampion		Topaz	
	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5
chlorogenic acid	245.5	179.5	271.9	218.9	132.4	118.3	130.1	121.2	363.5	306.3	425.8	402.9	186.5	155.7	98.9	89.0	47.1	49.8	117.9	90.7
p-coumaric acid derivatives	35.9	27.3	22.9	16.4	35.8	24.9	96.0	84.5	52.0	41.3	76.4	74.1	31.7	26.4	43.0	42.3	25.3	37.9	nd[b]	nd
caffeic acid	12.0	7.8	nd	nd	6.9	nd	21.0	18.0	nd	nd	nd	nd	nd	nd	16.1	12.6	nd	nd	nd	nd
hydroxycinnamic acids	**293.4**	**214.6**	**294.8**	**235.3**	**175.1**	**143.2**	**247.1**	**223.7**	**415.5**	**347.6**	**502.2**	**477.0**	**218.2**	**182.1**	**158.0**	**143.9**	**72.4**	**87.7**	**117.9**	**90.7**
catechin	50.2	28.0	30.0	36.3	52.5	23.2	67.2	64.3	64.7	42.2	61.7	57.9	42.8	26.7	67.4	65.5	63.2	32.8	45.1	44.3
epicatechin	392.1	354.5	331.7	229.4	294.5	249.8	376.9	313.8	398.5	380.8	502.1	477.4	459.6	403.4	438.8	268.1	415.8	499.8	382.5	289.2
procyanidin B1	208.2	177.8	155.0	133.5	266.6	244.3	176.4	154.3	184.5	186.5	283.4	219.3	196.6	182.5	166.8	148.2	207.7	188.7	154.7	140.4
procyanidin B2	145.3	102.0	79.2	77.1	65.3	54.5	83.5	83.1	98.3	90.6	125.5	106.2	91.2	78.2	87.1	71.5	102.1	138.8	88.3	80.2
other procyanidins	150.9	95.2	54.5	42.4	48.0	32.5	88.9	96.6	181.7	161.3	244.7	235.8	242.0	203.8	68.1	70.4	216.7	262.1	110.6	102.7
flavan-3-ols	**946.7**	**757.5**	**650.1**	**518.7**	**726.9**	**604.3**	**792.9**	**712.1**	**927.7**	**869.1**	**1217.4**	**1096.6**	**1032.2**	**894.6**	**928.2**	**623.7**	**1005.5**	**1122.2**	**781.2**	**656.8**
quercetin 3-rutinoside	12.6	6.4	12.5	10.6	16.6	16.5	6.8	6.2	18.8	12.6	8.6	7.9	22.4	18.3	43.5	22.5	7.0	6.6	15.3	11.7
quercetin glycosides	16.4	6.8	22.1	22.3	36.9	35.3	7.0	6.8	29.3	17.9	44.9	39.7	51.7	49.0	88.0	44.8	47.4	42.8	47.1	33.3
flavonols	**29.0**	**13.2**	**34.6**	**32.9**	**53.5**	**51.8**	**13.8**	**13.0**	**48.1**	**30.5**	**53.5**	**47.6**	**74.1**	**67.3**	**131.5**	**67.3**	**54.4**	**49.4**	**62.4**	**45.0**
phloridzin	80.8	63.9	nd	nd	nd	nd	nd	nd	25.2	19.7	29.8	25.2	35.4	29.1	nd	nd	nd	nd	nd	nd
phloretin derivatives	44.5	37.6	nd	nd	nd	nd	nd	nd	13.7	8.8	nd	nd	23.1	19.0	nd	nd	nd	nd	nd	nd
dihydrochalcones	**125.3**	**101.5**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**38.9**	**28.5**	**29.8**	**25.2**	**58.5**	**48.1**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
TPI (HPLC)[c]	1394.4	1086.8	972.6	786.9	955.5	799.3	1053.8	948.8	1430.2	1275.7	1802.9	1646.2	1324.5	1192.1	1117.7	834.9	1132.3	1259.3	961.5	792.5
TPC (FC)[d]	1602.1	1201.5	1075.4	863.9	1182.4	944.7	1157.1	1091.1	1325.5	1219.4	1849.1	1725.7	1461.9	1325.0	1188.7	1005.5	1279.6	1382.8	1119.0	913.5

[a] Data are the average of two measurement of 2 extracts for each sample . [b] Not detectable. [c] TPI is the sum of concentrations of all phenolic compounds; *F*-test cultivars ($P < 0.001$), *F*-test day ($P < 0.001$). [d] Total phenolic content measured by FC method in mg GAE 100 g^{-1} DW; *F*-test cultivars ($P < 0.001$), *F*-test day ($P < 0.001$).

Table 2. Concentrations (in μmol 100 g^{-1} DW) of Phenolic Compounds in Skin-On Apple Wedges from 10 Cultivars on Day 0 and after 5 Days of Storage at 2−4 °C in the Growing Season 2008[a]

	Alwa		Ariwa		Braeburn		Cortland		Gloster		Idared		Jonica		Rajka		Shampion		Topaz	
	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5
chlorogenic acid	249.2	182.5	309.9	270.9	173.6	123.8	229.0	191.3	560.6	461.8	306.5	276.3	247.6	192.8	106.8	98.9	65.7	85.6	119.8	116.2
p-coumaric acid derivatives	29.8	28.7	25.7	23.2	48.3	37.4	87.1	75.6	89.4	61.1	39.6	30.1	38.9	27.1	50.2	47.4	38.9	45.0	nd[b]	nd
caffeic acid	8.3	nd	nd	nd	2.1	4.2	28.8	26.6	nd	nd	nd	nd	nd	nd	16.0	14.6	nd	nd	nd	nd
hydroxycynammic acids	**287.3**	**211.2**	**335.6**	**294.1**	**224.0**	**165.4**	**344.9**	**293.5**	**650.0**	**522.9**	**346.1**	**306.4**	**286.5**	**219.9**	**173.0**	**160.9**	**104.3**	**130.6**	**119.8**	**116.2**
catechin	31.4	34.7	36.5	25.3	62.3	46.8	59.5	63.2	87.4	52.9	64.1	44.4	45.0	28.8	67.7	60.8	47.8	35.2	35.2	30.5
epicatechin	384.6	334.8	311.3	249.3	283.9	263.5	260.1	255.3	356.9	252.8	333.4	285.7	417.3	356.6	371.3	213.5	355.3	409.3	298.6	295.2
procyanidin B1	200.2	160.8	185.8	177.7	165.5	149.7	180.2	156.7	186.4	182.7	161.0	156.5	196.1	187.9	174.8	168.9	165.9	190.5	157.1	144.1
procyanidin B2	104.8	63.8	80.1	75.7	75.3	75.1	73.7	63.4	91.4	62.9	77.8	60.9	83.2	66.9	95.1	75.6	119.1	87.2	93.2	84.2
other procyanidins	137.1	66.6	76.1	65.8	129.0	70.3	78.4	77.5	214.6	153.7	160.4	126.0	263.1	189.9	130.7	68.4	175.0	259.2	127.8	112.2
flavan-3-ols	**858.1**	**660.7**	**689.8**	**593.8**	**716.0**	**605.4**	**651.9**	**616.1**	**936.7**	**705.0**	**796.7**	**673.5**	**1004.7**	**830.1**	**839.6**	**587.2**	**863.1**	**981.4**	**711.9**	**666.2**
quercetin 3-rutinoside	6.4	4.3	14.5	10.1	12.1	10.2	6.8	nd	9.1	8.4	20.3	8.3	10.3	7.4	7.3	5.0	10.2	8.5	21.3	21.0
quercetin glycosides	8.2	7.6	36.2	32.0	84.8	52.7	50.9	21.3	58.1	42.3	38.7	19.6	77.0	49.4	122.8	45.5	45.8	43.7	54.2	46.8
flavonols	**14.6**	**11.9**	**50.7**	**42.1**	**96.9**	**62.9**	**57.7**	**21.3**	**67.2**	**50.7**	**59.0**	**27.9**	**87.3**	**56.7**	**130.1**	**50.5**	**56.0**	**52.2**	**75.5**	**67.8**
phloridzin	66.5	52.7	28.2	25.5	20.5	14.5	18.0	nd	37.7	35.9	39.7	30.4	38.1	35.6	nd	nd	nd	nd	nd	nd
phloretin derivatives	37.6	32.4	nd	nd	21.4	nd	12.9	nd	19.0	17.3	nd	nd	28.1	27.8	nd	nd	nd	nd	nd	nd
dihydrochalcones	**104.1**	**85.1**	**28.2**	**25.5**	**41.9**	**14.5**	**30.9**	**nd**	**56.7**	**53.2**	**39.7**	**30.4**	**66.2**	**63.4**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
TPI (HPLC)[c]	1264.1	968.9	1104.3	955.5	1078.8	848.2	1085.4	930.9	1710.6	1331.8	1241.5	1038.2	1444.7	1170.1	1143.1	798.6	1023.4	1164.2	907.2	850.2
TPC (FC)[d]	1391.1	1141.2	1225.8	985.7	1237.5	1097.3	1247.0	1139.3	1600.6	1405.5	1491.0	1256.3	1575.1	1425.0	1216.1	1068.1	1166.0	1269.5	1181.2	1035.8

[a] Data are the average of two measurementd of 2 extracts for each sample. [b] Not detectable. [c] TPI is the sum of concentrations of all phenolic compounds; *F*-test cultivars ($P < 0.001$), *F*-test day ($P < 0.001$). [d] Total phenolic content measured by FC method in mg GAE 100 g^{-1} DW; *F*-test cultivars ($P < 0.001$), *F*-test day ($P < 0.001$).

The uniform nature of this degradation over two seasons for each could be a function of the unique biochemical reaction of each cultivar. To confirm the similar changes of each individual compound in both growing seasons a biochemical analysis is needed. In addition, since the apples were cut into the same segment sizes (wedges) in both years the surface exposed to oxygen was identical in area. The greatest loss in TPIs was for the Rajka cultivar (26.9 ± 3%) and lowest for Cortland apples (11.3 ± 3.1%.). In contrast to the other cultivars, the TPI for Shampion increased by 11.8 ± 1.5% (**Figure 1**).

As stated elsewhere flavan-3-ols were the most abundant polyphenolic group present in all samples, and levels of compounds from this group decreased significantly for nine of the ten cultivars over the 5 days storage at 2−4 °C. In the case of Shampion apples, flavan-3-ols were mostly responsible for the major increase in TPIs. Within the flavan-3-ol group (−)-epicatechin was the most abundant compound (and the most abundant overall) with levels ranging from 502.1 ± 10.1 μmol 100 g^{-1} dry weight (DW) for Idared apples (2007) to 260.1 ± 34.8 μmol 100 g^{-1} DW for Cortlands (2008) (**Table 1**, **2**). Łata (*32*) reported (−)-epicatechin to be a relatively stable compound in apples; however, in the present study levels of this compound decreased by 13.5 ± 16.5% on average over the five days of chill storage. This degradation must be at least in part due to PPO activity as several authors have reported that (−)-epicatechin is readily degraded by PPO (*33*, *34*). The degree of PPO activity might also

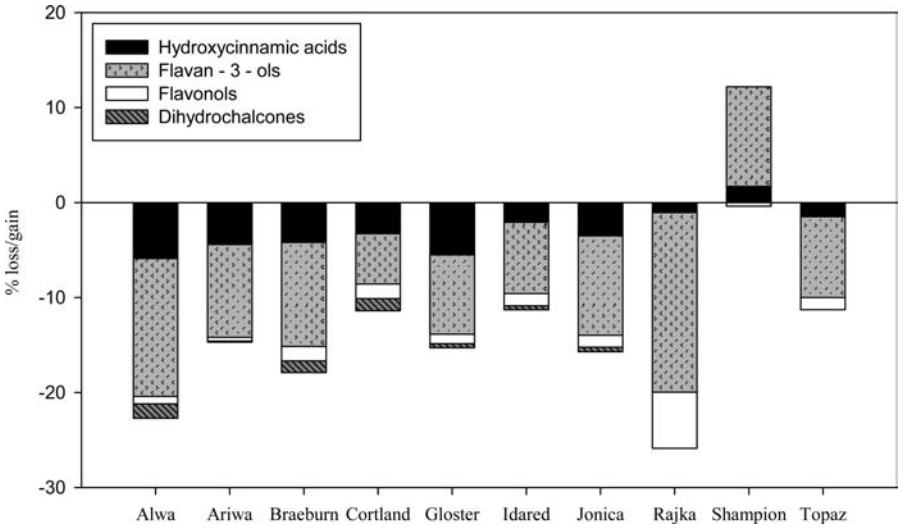

Figure 1. Percentage loss/gain after 5 days of storage at 2−4 °C of polyphenolic groups of skin-on apple wedges from ten different cultivars ('Shampion', 'Jonica', 'Gloster', 'Topaz', 'Ariwa', 'Rajka', 'Idared', 'Cortland', 'Alwa' and 'Braeburn') measured by HPLC method. Data averaged from sum of concentrations of all phenolic compounds detected in two growing seasons (2007 and 2008).

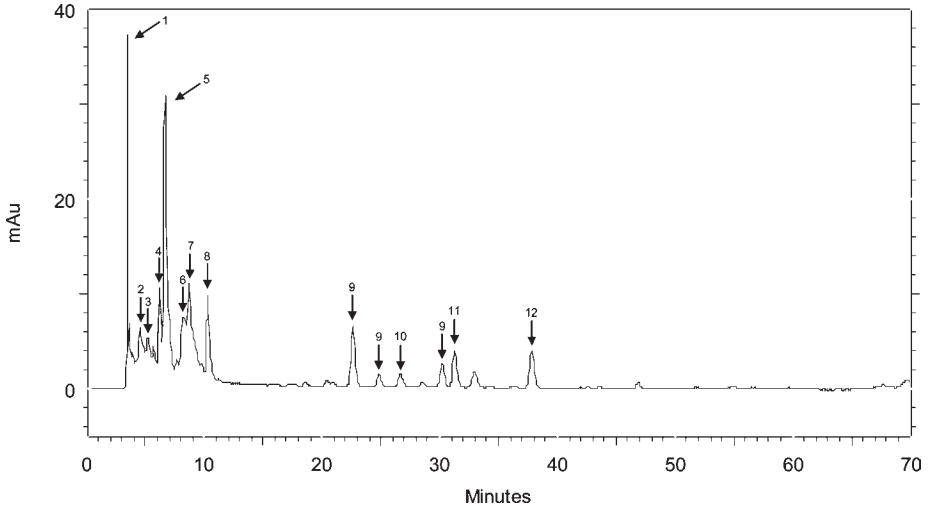

Figure 2. HPLC chromatogram at 280 nm of Braeburn (day 0, 2008) apple wedges methanolic extract. The peaks were identified as (1) procyanidin B1, (2) other procyanidin, (3) (+)-catechin, (4) procyanidin B2, (5) chlorogenic acid, (6) (−)-epicatechin, (7) caffeic acid, (8) *p*-coumaric acid derivative, (9) quercetin glycoside, (10) quercetin 3-rutinoside, (11) phloridzin, (12) phloretin derivative.

explain why it significantly ($P < 0.001$) increased for Shampion as it has been reported as this cultivar has low PPO activity (*35*). Other main flavan-3-ols are the procyanidins. Procyanidins such as B1 and B2 are potent antioxidants and therefore major contributors to the global antioxidant activity of apples (*36*). These compounds were present at appreciable levels in the cultivars examined in the present study. For example in 2007 day 0 values for procyanidin B1 ranged from 283 ± 23.6 μmol 100 g^{-1} DW for Idared to 154.7 ± 23.8 μmol 100 g^{-1} DW for Topaz (**Table 1**). In 2008, values ranged from 200.2 ± 11.4 μmol 100 g^{-1} DW for Alwa apples to 157.1 ± 23.8 μmol 100 g^{-1} DW for Topaz on day 0. The highest level of procyanidin B1 was detected after chill storage in Braeburn apples in 2007 (244.3 μmol 100 g^{-1} DW). In comparison to other compounds such as (−)-epicatechin, procyanidin B1 and B2 were more stable to chill storage with average losses of 10.4 ± 2.9% occurring in the present study. Several other compounds with UV spectra 99% similar to that of procyanidin A2 were detected. These were presumably glycosidic derivatives of this compound; however, further work would be required to confirm their identity. Therefore these compounds are reported in **Table 1** and **2** as "other procyanidins" and quantified by comparison to standard curves derived from authenticated standard of procyanidin A2.

In agreement with other studies (*25*), hydroxycinnamic acids were the second most abundant group present in apples. These compounds were also responsible for the second largest loss/gain of the fresh-cut apple wedges after 5 days of storage (**Figure 1**). Alwa had the highest loss (5.9 ± 0.3%) while Rajka had the lowest loss over 5 days of storage. Similar to the behavior reported for flavan-3-ols in Shampion apples, hydroxycinnamic acids increased by 1.7 ± 0.6% in this cultivar during chill storage for 5 days. Oszmiański et al. (*35*) reported that chlorogenic acid (a hydroxycinnamic acid) is a good substrate for PPOs. Therefore reductions in levels of hydroxycinnamic acids may be due enzymatic degradation of this compound. As the results in **Table 1** and **Table 2** show, chlorogenic acid was the most abundant of the hydroxycinnamic acids. Other authors have also reported that this compounds is one of the major polyphenols present in apples (*9*, *11*). There were large variations in the level of chlorogenic acid between cultivars. For example in 2007,

cholorogenic acid values ranged from from 425.8 ± 47.9 μmol 100 g^{-1} DW (Idared) to 41.7 ± 0.7 μmol 100 g^{-1} DW (Shampion) on day 0 (**Table 1**). This highest level of this polyphenol was detected in 2008 in Gloster apples on day 0 (560.6 ± 40.5 μmol 100 g^{-1} DW). The average degree of degradation of chlorogenic acid was similar that of the flavan-3-ols (12.1 ± 1.2%). *p*-Coumaric acid derivatives were found to be the second most abundant compounds in the hydroxycinnamic acids group. Several studies have shown these compounds are more likely to be *p*-coumaroylquinic acids (*25*, *27*). It was present in nine of the ten cultivars but could not be detected in Topaz in either season. There was no significant difference in the loss of *p*-coumaric acid derivatives between the seasons. Gloster had the highest loss at 26.7 ± 5.6% and Rajka the lowest at 4 ± 1.6% while Shampion gained an average of 30 ± 9.7%. In comparison to other compounds, caffeic acid was present at relatively low levels and was in fact only detected in Alwa, Braeburn, Cortland and Rajka cultivars in 2007 and 2008. In Braeburn (2007) and Alwa (2008) this compound was absent after chill storage indicating that it was fully degraded after storage.

Flavonols contributed approximately 5% to the overall TPIs. This figure varied significantly between cultivars ($P < 0.001$) and growing seasons ($P < 0.001$). Rajka was found to have the highest level of flavonols in both years with 131.5 ± 6.6 μmol 100 g^{-1} DW in 2007 and 130.1 ± 7.6 μmol 100 g^{-1} DW in 2008. This cultivar also exhibited the highest loss of flavonols in both seasons with an average loss of 54.8%. In contrast to Rajka, flavonols were a relatively minor constituent in the other nine cultivars. Since flavonols are usually present in the highest level in the peel of the apple (*37*), it is not surprising that they were a relatively minor constituent as the peel contributed approximately only 5% of each wedge.

Dihydrochalcones were present in Alwa, Ariwa, Braeburn, Cortland, Gloster, Idared, Jonica cultivars in 2008 and Alwa, Gloster, Idared, Jonica apples in 2007. Dihydrochalcones were relatively stable during storage with losses ranging from 1.5 ± 0.3% for Alwa to 0.1 ± 0.06% for Ariwa. Alwa had the highest levels of dihydrochalcones in both seasons which were mostly accounted by high levels of phloridzin. Phloridzin was found more present at higher levels in most cultivars than its derivatives, and if detected phloretin derivatives were 100% degraded following 5 days chill storage for some cultivars. Similar to the case for anthocyanins and flavonols, dihydrochalcones were not present at high levels as they have been reported to be present mostly in the peel and not in the flesh (*15*).

In summary all ten cultivars showed significant differences ($P < 0.001$) in the individual polyphenols. By characterizing the cultivars in terms of TPIs from 2007 and 2008 and their overall polyphenols on days 0 and 5, they can be ranked in the following decreasing order: Gloster > Idared > Jonica > Alwa > Shampion > Cortland > Rajka > Ariwa > Braeburn > Topaz. Growing season also had a significant impact ($P < 0.001$) on most of the cultivars as previously reported, but especially on the group of total flavan-3-ols.

Total Phenolics by FC Assay. Total phenolic content (TPC) determined by the Folin−Ciocalteu assay of the ten apple cultivars for two storage days (0 and 5) are presented in **Table 1** (2007) and **Table 2** (2008). Significant differences ($P < 0.001$) were found between the ten cultivars ($P < 0.001$) in each season; however, there was no significant difference between the two years. In 2007 the amount of total phenolics ranged from 1849.1 ± 194.3 mg GAE/100 g DW (Idared) to 1075.4 ± 144.9 mg GAE (Ariwa) on day 0 and from 1725.7 ± 193.2 mg GAE/100 g DW (Idared) to 863.9 ± 114.2 mg GAE (Ariwa) on day 5. The largest loss in TPC was found in Alwa with 24.8 ± 2.8% (400.6 ± 83.6 mg GAE/100 g DW) and smallest loss of 5.6 ± 1.9% (123 ± 54.4 mg GAE/100 g DW) for Idared after 5 days storage in 2007. TPC values for Shampion wedges increased (8.1 ± 3.9%) over the 5 days storage from 1279.6 ± 60.8 to 1382.0 ± 15.8 mg GAE/100 g DW. As discussed above levels of individual phenols in Shampion apples also increased during storage. In 2008 the largest loss in TPC was detected in Ariwa apples with 19.5 ± 1.3% (240.1 ± 37.1 mg GAE/100 g DW) and smallest loss of 8.2 ± 2.5% (107.7 ± 30.3 mg GAE) over 5 days storage. Overall the TPC values correlated well to the TPIs analyzed by HPLC in 2007 ($r = 0.93$) and 2008 ($r = 0.88$). If characterized in terms of TPCs from 2007 and 2008 and their overall total phenolics on day 0 and 5, the cultivars can be ranked in the following decreasing order: Gloster > Jonica > Idared > Alwa > Shampion > Cortland > Braeburn > Rajka > Topaz > Ariwa. The slight differences between the TPI and the TPC measurements were expected as the FC method also measures non-polyphenolic compounds such as ascorbic acid (*38*, *39*).

In conclusion, the total polyphenolic contents, as well as the major groups and individual compounds, varied significantly among the ten cultivars and the two growing seasons. Storage of fresh-cut apple wedges resulted in losses of polyphenols for nine of the ten cultivars and in a gain in the case of Shampion. Storage resulted in similar changes in polyphenolics in both growing seasons suggesting that degree of change was related to the concentration in the fruit and the intrinsic biochemistry of the cultivar. Gloster, Idared, Jonica, Alwa and Shampion were highest in TPIs and TPCs and contained the largest variety of compounds also. Therefore these cultivars could be recommended as core products in fresh-cut fruit products in terms of contribution to health benefits. However, Cortland, Braeburn, Rajka, Topaz and Ariwa did not score as high as the other five cultivars but they still showed a large amount of polyphenols if compared to other fruits.

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